Proceedings of the Nutrition Society (2008), **67 (OCE)**, E214 doi:10.1017/S002966510800846X

A Scientific Meeting was held at the West Park Conference Centre, Dundee, Scotland, on 27 and 28 March 2008

Acid survival of *Escherichia coli*; how dead is dead?

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Escherichia coli is a significant food-poisoning pathogen and recent outbreaks have caused increased concerns from experts in the field of nutrition and food science. Population diversity demonstrated by commensal *E. coli* when growing exponentially in a defined medium is most evident in the formation of tailing populations of surviving organisms when the population is subjected to extreme stress[(1)]. The present study describes the fluorescence-activated cell sorting (FACS)-based analysis of *E. coli* acid survival.

Acid killing was performed on purified commensal *E. coli* strain J1. Culture of *E. coli* strain J1 in mid-exponential phase was subjected to extreme pH stress at pH 3, with *E. coli* strain Frag 1 as control. A viable colony count was performed to estimate the rate at which cells die. Cell viability was assessed using a BacLight kit (Invitrogen Ltd, Paisley, UK). J1 cells were stained using a BacLight kit containing SYTO9 and propidium iodide stains (Invitrogen Ltd). For FACS analysis fully-stained J1 cell populations were prepared and incubated in a medium at either pH 7 or pH 3 for different time intervals. Samples were centrifuged, resuspended in a minimal medium (pH 7) without growth supplements and scanned using an FACS DIVA cell sorter (Becton Dickinson UK Ltd, Oxford, Oxon., UK). Windows were defined based on controls. Once a window had been defined to separate cells from the population based on viable and non-viable properties, cells were allowed to rest anaerobically in the minimal medium. After a 1 h resting period cells were re-scanned.

Acid-killing experiments revealed that *E. coli* strain J1 survives extreme pH stress (pH 3) better than strain Frag 1, and a surviving population of J1 was identified. From FACS analysis J1 cells incubated in a medium at pH 7 or pH 3 were highly fluorescent with SYTO9, with cells incubated at pH 3 showing progressive loss of SYTO9 fluorescence with increasing incubation period. When a second scan was conducted at approximately 60 min after resuspension in the minimal medium at pH 7, cells incubated at pH 3 for 10 min and rested for 60 min at pH 7 were observed to divide into two populations, one population shifted towards the viable end with higher SYTO9 fluorescence than before, while the other population remained in the same position. Cells incubated at pH 3 for 20 min and rested for 60 min at pH 7 were also observed to divide into two populations; one population showed a reduced SYTO9 fluorescence and hence shifted towards the non-viable end, while the other population remained in the same position. Cells incubated at pH 3 for 30 min and 40 min before being rested for 60 min at pH 7 showed a shift towards the non-viable end. No propidium iodide fluorescence was observed.

The results suggest that cells do die during incubation in the medium at pH 3; however, some cells are more resistant and may be able to recover and repair DNA with time when pH is restored. Fewer cells recover with increasing period of incubation at pH 3. As there may be recovering cells that are perceived to be dead in current food diagnostic procedures involving the use of the BacLight kit, these results may help enhance current procedures to ensure that food products are free from contamination.

1. Booth IR (2002) *Int J Food Microbiol* **78**, 19–30.